Delayed Onset of Epidermal Differentiation in Psoriasis

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In normal epidermis, as previously reported, the first signs of differentiation occur within the basal layer in a subpopulation of keratinocytes that start to express K1 and K10 "suprabasal" keratin transcripts (20–30% of the basal cells) and proteins (5–10% of the basal cells). We found that in psoriatic lesions, the basal layer was devoid of cells expressing these early differentiation markers. This was already the case at the periphery of the lesions, where epidermis, although slightly acanthotic, still completes the keratinization process. In the center of the lesions, not only the basal layer, but also several rows of suprabasal cells, were negative for keratin K10 transcripts or protein. Moreover, the upper nucleated layers of involved epidermis were also devoid of K10 keratin transcripts or proteins. In normal epidermis, as previously reported, transcripts for the "basal" K5 keratin were mainly restricted to the basal layer, whereas the protein persisted in a few suprabasal layers. We found that in psoriatic epidermis, K5 keratin transcripts persisted in several suprabasal layers up to the level where K10 keratin transcripts appeared.

These data, although not contradictory with previous reports showing a reduction of K1–K10 keratins and other differentiation markers in psoriasis, demonstrate that these quantitative changes are in fact the result of major qualitative differences in the distribution of these markers in psoriatic versus normal skin. Our results indicating that the onset of differentiation is delayed in psoriasis show that, contrary to conclusions accepted so far, not only the suprabasal compartment, but also the basal one, is abnormal in psoriatic epidermis. *J Invest Dermatol 98:902–910, 1992*

Psoriasis is a rather common disease, but of unknown etiology. Genetic studies have pointed out a hereditary component compatible with an autosomal dominant inheritance with a penetrance of approximately 60% [1]. As an attempt to identify the primary cellular target of the psoriatic trait, many histopathologic studies have been focused on the earliest symptoms appearing during the development of a psoriatic skin lesion in order to define precisely their sequence of appearance. Although it cannot be demonstrated that epidermis is the site that is primarily affected in psoriasis, it is unquestionable that epidermal symptoms are very precocious during the development of a skin lesion and that they are specific to this disease [2].

The most renowned histologic features of psoriatic epidermis are *agranulosis* and *parakeratosis,* i.e., the absence of granular layers and of normal anucleated cornified layers. On the contrary, spinous layers are considered to be hyperplastic but abnormal in psoriasis (*acanthosis* and *papillomatosis*). Differentiation markers of the suprabasal compartment such as K1–K10 keratins are reduced, whereas the "hyperproliferative" K6–K16 keratins are induced in psoriasis [3–8]. As for the basal layer, it is considered to be normal according to several morphologic and immunohistologic criteria [3,4,9]. The current interpretation of these histologic and biochemical findings is that, in psoriatic epidermis, only the last steps of differentiation are altered—in other words that the differentiation program starts normally, but is then "truncated."

However, we show in the present work that in psoriatic epidermis, the differentiation pattern is abnormal from the very beginning. Although in normal epidermis the onset of differentiation occurs within the basal layer in a subpopulation of cells that have started to synthesize the K1 and K10 "suprabasal" keratins before detaching from the basement membrane [10–13], we found that, in psoriatic epidermis, cells expressing K10 transcripts and proteins can be detected only in the suprabasal compartment. This delay in the onset of differentiation increases from the periphery towards the center of a lesion where the first cells synthesizing K10 keratin appear several rows above the basal layer. Moreover, contrary to what is observed in normal epidermis, the upper nucleated layers of psoriatic epidermis contain no K10 keratin mRNA or protein. Thus, the delay in the onset of K10 keratin synthesis and its arrest in the upper nucleated (parakeratotic) layers are probably both responsible for the previously reported reduction in expression of K10 (and K1) keratins in psoriasis as compared to normal epidermis.

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Abbreviations:
DTT: dithiothreitol
EDTA: ethylenediaminotetraacetate
FITC: fluorescein-isothiocyanate
IgG: immunoglobulin G
MoAB: monoclonal antibody
PBS: phosphate-buffered saline
PIPES: piperazine-N, N′-bis α-ethanesulfonic acid
PoAb: polyclonal antibodies
SSC: saline sodium citrate
UTP: uridine triphosphate

MATERIALS AND METHODS

Skin Samples Normal human skin consisted of surgical samples obtained after mammary reduction (three samples) or face-lifting (four samples). Biopsies of psoriatic skin were generously given by Prof. L. Juhlin (Sweden) from three patients with a long history of psoriasis vulgaris. Punch biopsies (4-mm diameter) were taken from different areas of the psoriatic lesions: periphery, edge, and center of

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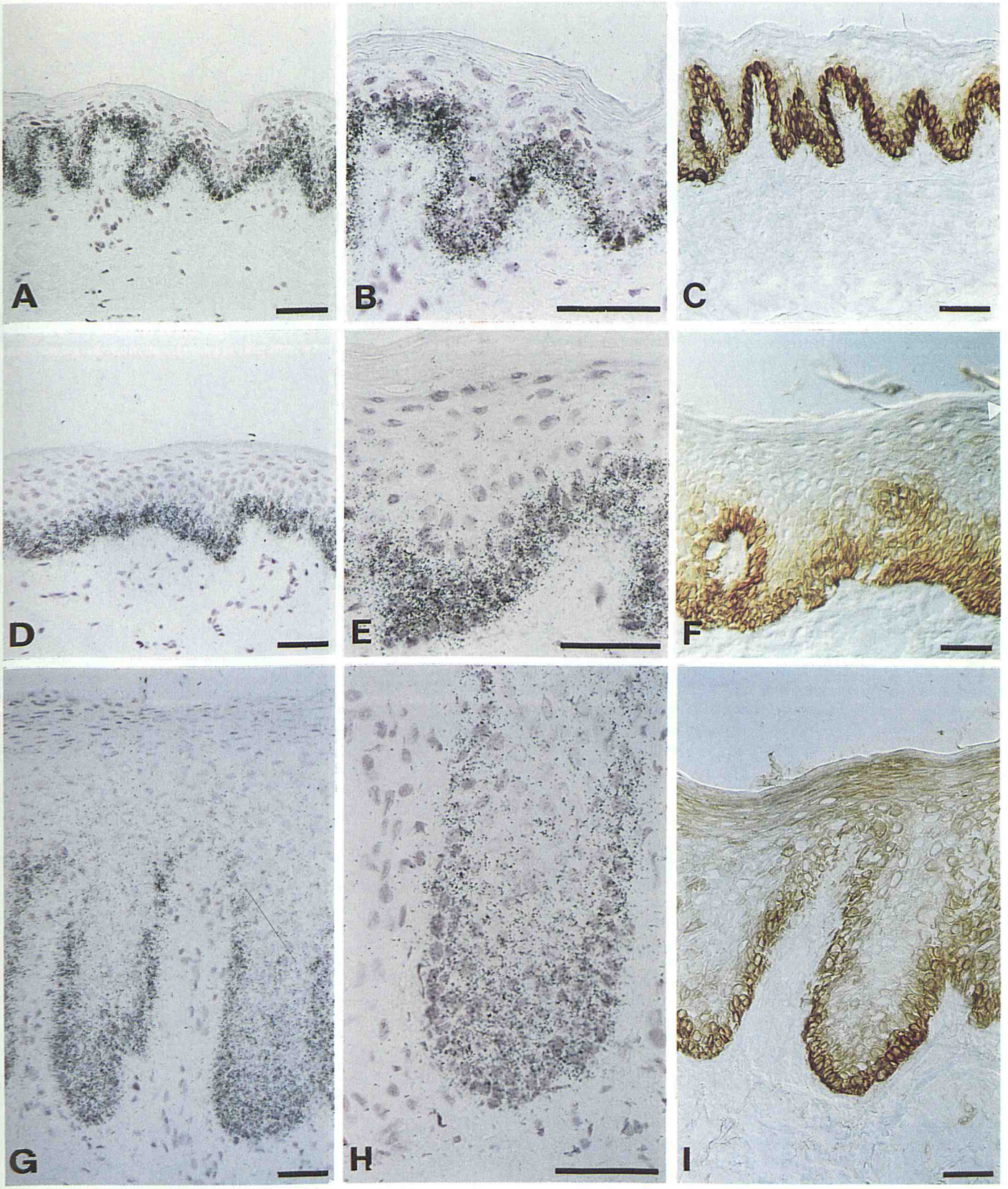
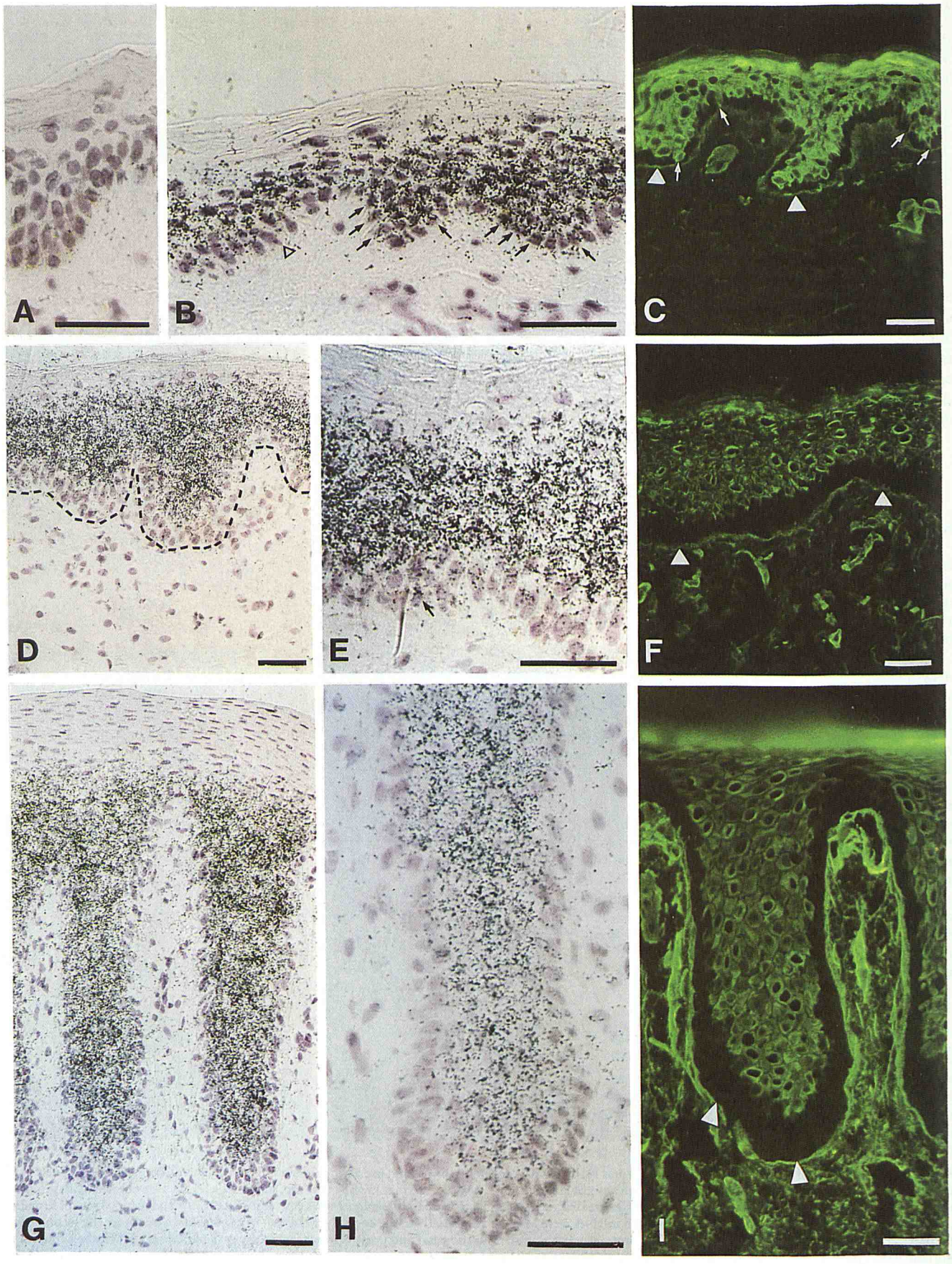


Figure 1. Distribution of K5 "basal" keratin mRNA and K14 "basal" keratin in normal and psoriatic epidermis. In normal epidermis *(A,B,C)*, the K5 probe hybridized predominantly with the basal cells although a slight labeling could be observed in some first row suprabasal cells *(A,B)*. Immunoperoxidase labeling with the FB1 anti-K14 MoAb of an adjacent section *(C)* revealed a strong signal in the basal cells and a weaker staining in the first and second rows of suprabasal cells. At the periphery of a psoriatic lesion *(D,E,F)*, K5 mRNA were localized essentially in the basal layer but also in the first and sometimes the second row of the suprabasal cells *(D,E)*. In the same region K14 proteins were abundant in the basal and present in several suprabasal cell layers *(F)*. In the center of the psoriatic lesion *(G,H,I)*, K5 transcripts were detected in the lower part of epidermis, including not only the basal layer, but several suprabasal layers *(G,H)*. In those areas, the FB1 anti-K14 MoAb showed an intense labeling in the basal and parabasal cells of the rete ridges and a weaker immunoreactivity in the remaining epidermis *(I)*. *Bar*, 50 μm.


A
B
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a psoriatic plaque as previously described by Parent et al [2]. Uninvolved skin outside of the lesions was also biopsied. Papillomatous skin was biopsied in the elbow area of a volunteer normal subject. Samples were frozen in liquid nitrogen immediately after biopsy and stored at −70°C until use.

Immunolabelings Skin samples frozen in liquid nitrogen were embedded in Tissue Tek (Miles, USA), and cut in 5-μm vertical cryostat sections. After air drying, the sections were rinsed in phosphate-buffered saline pH 7.2 (PBS, Biomérieux Laboratories, France) and immunolabeled at room temperature.

Antisera: Mouse monoclonal antibody (MoAb) to human K10 keratin (RKSE60) was purchased from Sanbio Laboratories (Netherlands). Mouse MoAb to human collagen IV was purchased from Serotec (England). Rabbit polyclonal antibodies (PoAb) to human involucrin were purchased from Biomedical Technologies Inc. (USA). Mouse MoAb to human K14 keratin (FB1) was a generous gift from Dr. D. Parent (Belgium). Antisera were diluted in PBS as follows: 1/10 for anti-K10, 1/100 for anti-collagen IV, and 1/2 for anti-involucrin. FB1 anti-K14 MoAb was used undiluted.

Double Labeling with Anti-K10 MoAb and Anti-Collagen IV MoAb: Sections were incubated with both antibodies for 30 min, washed with PBS, incubated with rabbit anti-mouse IgG FITC or Rhodamine conjugate (Dako, Denmark) (dilution: 1/300) for 30 min, washed again with PBS, and mounted.

Triple Labeling with K10 MoAb, Collagen IV MoAb, and Involucrin PoAb: Sections were incubated in a mixture of K10 MoAb and collagen IV MoAb for 30 min, washed in PBS, incubated with a goat anti-mouse rhodamine-conjugated IgG (dilution 1/100) (Tago, USA), washed in PBS, incubated with involucrin PoAb for 30 min, and washed again with PBS. Sections were then incubated with a swine anti-rabbit IgG FITC conjugate (Dako, Denmark) (dilution 1/300) for 30 min, washed in PBS, and mounted.

Immunoperoxidase Staining with Anti-K14 MoAb: Sections were incubated with FB1 anti-K14 MoAb for 30 min, washed with PBS, incubated with a goat anti-mouse biotinylated IgG (1/200 in PBS) for 90 min, washed with PBS, and incubated with an avidin-peroxidase complex (1/80 in PBS for 90 min) (Vectastain Kit, Vector Laboratories, USA). Sections were rinsed with PBS and revealed with 3-3′diaminobenzidine (Sigma) for 15 min, rinsed, and mounted.

In Situ Hybridization

Keratin K10 and K5 cRNA Probes: K10 and K5 cDNA were isolated by Darmon et al [14] and Galup and Darmon [15], respectively. Both K10 and K5 partial cDNA correspond to the 3′ portion of published sequences. The K10 insert matches the first 230 nucleotides of the highly conserved 3′ noncoding region of exon 8 [14,16]. The K5 insert encompasses the last 3′ 500 nucleotides published by Galup and Darmon [15]. Both cDNA were subcloned into the Pst1 site of the pBluescript IISK+ vector (Stratagene, USA). Constructs were verified by double-stranded DNA sequencing (Amersham Multiwell Sequencing Kit) according to the dideoxy-termination chain method [17]. Specificity of probes was checked by Northern blot analysis (data not shown) using RNA extracted from primary cultures of human keratinocytes in high-calcium MCDB153 (KBM, Clonetics, San Diego, USA).

In both cases, RNA probes were generated by in vitro transcription from either the T3 (sense strand) or the T7 polymerase promoters (antisense strand) in the presence of ^{35}S UTP (1000 Ci/mMol, Amersham). After column purification and ethanol precipitation, labeled probes were dissolved at 5×10^7 cpm/ml in 50% deionized formamide, 50% 2× hybridization buffer (40 mM piperazine-N, N′-bis 2-ethanesulfonic acid [PIPES] pH 7.4, 1.2 M NaCl, 20% dextran sulfate, 200 mM dithiothreitol (DTT), and 250 μg/ml of sheared salmon sperm DNA and 500 μg/ml yeast tRNA).

Processing of Tissue Sections for In Situ Hybridization: In situ hybridization was performed mainly according to Angerer et al [18]. Six-micrometer vertical cryostat sections were placed on 3-aminopropyltriethoxysilane-pretreated glass slides (Digene Diagnostics Inc., USA) and fixed in 4% (w/v) paraformaldehyde in PBS, rinsed in PBS, and stored in 70% ethanol at 4°C until use. Before hybridization, sections were rinsed in 300 mM NaCl, 30 mM sodium citrate (2 × SSC), acetylated in 0.25% acetic anhydride in 0.1 M triethanolamine pH 8.0 for 15 min at room temperature, rinsed in 2 × SSC, dehydrated in 70% ethanol, 100% ethanol, and air dried. Twenty microliters of probe were added per section. Sections were covered with a coverslip and sealed with rubber cement. Hybridization was done overnight at 52°C in a water bath. After removing the rubber cement and the probe by a 2 × SSC wash, slides were rinsed in 4 × SSC containing 10 mM DDT for 1 h at room temperature, washed twice in 2 × SSC, 50% formamide, and 10 mM DDT at 52°C for 30 min, and washed in 2 × SSC. All slides were immersed in RNase buffer (10 mM Tris HCl pH 8.0, 1 mM EDTA, 0.3 M NaCl), and treated for 30 min at 37°C in RNase A solution (40 μg RNase A/ml of buffer). After incubation in 2 × SSC, 50% formamide for 30 min at 65°C, slides were washed in 2 × SSC, dehydrated in 70% ethanol, 100% ethanol, and air-dried before autoradiography. Autoradiography was performed with Amersham LM1 emulsion. After 10 h, 3 d, and 1 week of exposure, slides were revealed and counterstained with Mayer's hemalun solution (Merck). Bright field micrographs were taken with a Zeiss Axiophot microscope.

RESULTS

Distribution of mRNA and Proteins Corresponding to the "Basal" K5/K14 Keratin Set in Normal Versus Psoriatic Epidermis To study the distribution of keratin K5 mRNA, skin sections were hybridized in situ with a riboprobe specific for this keratin (see *Materials and Methods*). In normal human epidermis, the label predominated largely in the basal layer, although a slight labeling could be detected in some cells of the first suprabasal layer (Fig 1*A,B*). The anti-K14 antibody made available to us (FB1, see *Materials and Methods*) labeled very strongly the basal layer of normal epidermis, much less intensely the spinous layers, and not at all the granular and cornified layers (Fig 1C). This result differs slightly

Figure 2. Distribution of K10 mRNA and K10 keratin in normal and psoriatic epidermis. In normal human skin *(A,B,C)*, K10 mRNA *(B)* are very abundant in all nucleated suprabasal layers but silver grains can be also observed in about 20–30% of the basal cells *(arrows)*. The *open triangle* points to a negative basal cell; the nucleolus that is stained purple by hemalum must be distinguished from silver grains that appear in black. *(A)*, negative control with sense riboprobe. Immunofluorescence detection of K10 keratin *(C)* shows a strong expression in the cytoplasm of the suprabasal layers including the granular layer. However, about 5–10% of the basal cells are also labeled *(arrows)*. These V-shaped transitional cells are in the process of detaching from the basement membrane. *White triangles,* basement membrane labeled with an anti-type IV collagen antibody. At the periphery of psoriatic lesions *(D,E.F)*, K10 transcripts are strongly expressed in most suprabasal layers and more weakly in the last nucleated layers. As in normal epidermis, no labeling was found in the stratum corneum. Contrary to our observations in normal epidermis, the basal layer was almost totally free of K10 transcripts *(D,E)*. Only few cells showed a few silver grains *(arrow)*. K10 keratin could not be detected in the basal layer, but was abundant in all nucleated suprabasal layers *(F)*. In the center of psoriasic lesions *(G,H,I)*, K10 transcripts were abundant in the central portion of the rete ridge areas, but the basal layer and even the first and second suprabasal rows were totally devoid of silver grains *(G,H)*. This was also the case of the parakeratotic horny layer. Basal cells and even the first and second rows of suprabasal cells did not show any labeling with anti-K10 antibody *(I)*. However, K10 keratin was abundant in the center of the rete ridge area. *Bar,* 40 μm.

from those obtained with another anti-K14 antibody, which labeled heavily the basal layer and rather strongly all nucleated suprabasal layers [4]. This discrepancy suggests that the epitope recognized by the FB1 anti-K14 antibody is partially masked or corresponds to a portion of the protein that is degraded in the suprabasal layers. Altogether, the above results indicate that in normal epidermis, the amount of K5 transcripts is high in the basal layers and decreases dramatically in the suprabasal layers, whereas K14 protein persists in a few suprabasal layers, although in reduced amounts compared to the basal layer. Results similar to ours reported previously in normal human and murine skin with K5 and K14 polynucleotide probes and antibodies [4,13,19] also show that both partners of the basal keratin set, K5 and K14, are coordinately expressed in the basal layer and then down-regulated.

In psoriatic epidermis, we consistently found that the K5 probe labeled not only the basal layer, but also several suprabasal layers. This was already the case at the periphery of the lesions where epidermis is histologically almost normal (Fig 1*D,E*), but it was particularly obvious in the center of the lesions (Fig 1*G,H*). At the periphery of the lesions, the FB1 anti-K14 antibody labeled more layers than in normal epidermis (Fig 1*F*). In the center of the lesions, this antibody labeled the entire epithelium (Fig 1*I*), although the label in the basal and parabasal cells was much stronger than in the suprabasal layers. By studying serial sections both perpendicular and parallel to the surface of epidermis (data not shown), we checked that this characteristic distribution of K5 messages and protein was true and not due to particular angles of sections. The above results are consistent with the hypothesis that, in psoriatic epidermis, the down-regulation of K5/K14 mRNA and proteins occurs more tardily than in normal epidermis. The persistence of K14 keratin in the outer layers of psoriatic epidermis was already mentioned by Stoler et al [4].

Distribution of mRNA and Proteins Corresponding to the "Suprabasal" K1/K10 Keratin Set in Normal Versus Psoriatic Epidermis In normal epidermis, the K10-specific riboprobe labeled not only all nucleated suprabasal layers, but also a large number of basal keratinocytes (approximately 20–30%) (Fig 2*B*). Moreover, the anti-K10 antibody labeled not only all suprabasal layers, but also a significant number (approximately 5–10%) of basal keratinocytes (Fig 2*C*). These keratinocytes consistently exhibited an elongated shape, which suggested that they were starting to detach from the basement membrane. The above results show that in a normal epidermis, a subpopulation of basal keratinocytes is already engaged in the differentiation process. These transitional keratinocytes have previously been recognized with anti-K1 and anti-K10 antibodies, and specific K1 and K10 polynucleotide probes both in human and murine epidermis [10–13].

Most interestingly, transitional basal keratinocytes were never found in involved psoriatic epidermis. Even at the periphery of the lesions, the basal layer contained very few cells labeled by the K10 riboprobe (Fig 2*D,E*). In the center of the lesions, not only the basal layer, but also the first suprabasal layer, was completely devoid of label (Fig 2*G,H*). In fact, in these areas, hybridization with the K10 probe seemed to start where hybridization with the K5 probe ceased (compare Fig 1*G,H* with Fig 2*G,H*). At the periphery of the lesions, no staining with the anti-K10 antibody could be detected in the basal layer (Fig 2*F*), whereas in the center of the lesions, not only the basal layer, but also several suprabasal layers, remained unstained by this antibody (Fig 2*I*). Staining of serial sections both parallel and perpendicular to the surface of epidermis (not shown) was performed to eliminate the possibility of misinterpretation due to particular angles of sections.

Altogether, the above results show that in psoriatic epidermis, the onset of K10 transcription and translation is delayed. This delay is already apparent at the periphery of the lesions, but most obvious in their center. Moreover, the abnormal abundance of K5 messages in suprabasal layers of these areas confirms that the undifferentiated phenotype persists well above the basal layer.

Uninvolved psoriatic skin and papillomatous skin from normal subjects showed a pattern of distribution of K5 and K10 transcripts and of K14 and K10 keratins similar to that of normal skin (data not shown).

In Psoriatic Lesions, the Pattern of Keratin Expression Is Not the Same in the Rete Ridges and in the Juxtapapillar Areas In the center of the psoriatic lesions, the pattern of keratin expression displays the following features: in the rete ridges, basal cells and several layers of suprabasal cells (up to 3) express K5 messages (Fig 3*B*), then K10 messages appear (Fig 3*F*) and persist up to the parakeratotic nucleated stratum corneum (Fig 2*G*). The FB1 anti-K14 antibody labels the entire thickness of the epithelium with a predominance in the basal and parabasal layers and a weak and non-homogeneous staining in the upper layer (Fig 3*A*), whereas K10 keratin appears several rows above the basal layer (Fig 3*E*) and persists up to the parakeratotic stratum corneum (Fig 2*I*).

In the juxtapapillar areas, the labeling with both the K5 and the K10 riboprobe was fainter than in the rete ridges. The labeling with the K5 probe was weak in the basal layer and very weak but still detectable in the first 3–4 rows of suprabasal cells (Fig 3*D*). In the region facing the very tip of the dermal papilla, the K10 probe did not label the basal layer, labeled weakly the first suprabasal layer (Fig 3*H*), but not the upper part of epidermis (parakeratotic stratum corneum). The FB1 anti-K14 antibody labeled weakly, but homogeneously, the totality of the juxtapapillary epidermis (Fig 3*C*). But, with the anti-K10 antibody, almost no staining could be detected in the region facing the very tip of the dermal papilla (Fig 3*G*).

Altogether, the above results show that both in the rete ridges and in the juxtapapillary areas, not only is the onset of K10 keratin synthesis delayed, but its synthesis stops in the upper nucleated layers, which form the parakeratotic stratum corneum. Thus, in the region facing the dermal papilla, differentiation seems to be even more affected than in the rete ridges, because the parakeratotic stratum corneum starts only a few layers above the basal layer. Moreover, it must be noted that in the juxtapapillary area, K5 keratin expression is also weak, even in the basal layer. These results contrast with the fact that K16 "hyperproliferative" keratin is abundant in this area [4].

Distribution of Involucrin in Normal Versus Psoriatic Epidermis It is well established that involucrin is expressed precociously in psoriatic epidermis [20–22], an observation that contrasts with our present finding that K10 keratin expression is delayed. Therefore, it seemed interesting to compare the distribution of involucrin with that of K10 keratin in the whole series of normal and psoriatic samples used in this study. Figure 4 shows double-staining experiments with anti-K10 MoAb and anti-involucrin serum. In normal epidermis (Fig 4*A,B*), involucrin appears in the granular layers, i.e., several layers above the basal layer and K10 keratin appearance. In psoriatic epidermis, at the periphery of the lesions, involucrin was expressed more precociously in the upper spinous layers, but still several layers above the basal layer and above K10 keratin (Fig 4*C,D*). In the center of the psoriatic lesions, the staining pattern was, as expected, very different in the rete ridge and juxtapapillar areas. In the rete ridge areas (Fig 4*E,F*), involucrin was expressed later than K10 keratin, several layers above the basal layer, whereas in the juxtapapillar area—a region where K10 keratin is very scarce or even absent—involucrin was directly suprabasal (Fig 4*G,H*).

DISCUSSION

The fact that psoriatic epidermis contains lower amounts of K1/K10 keratins than normal epidermis has already been reported by us and others [3]. In the present report, which, to our knowledge, is the first one using in situ hybridization of K10 transcripts in psoriasis, we show that the onset of K10 keratin synthesis is delayed in involved tissue. Actually, even at the periphery of the lesions, where epidermis is almost normal from a histologic point of view, the basal layers were found to be devoid of cells expressing K10 transcripts or

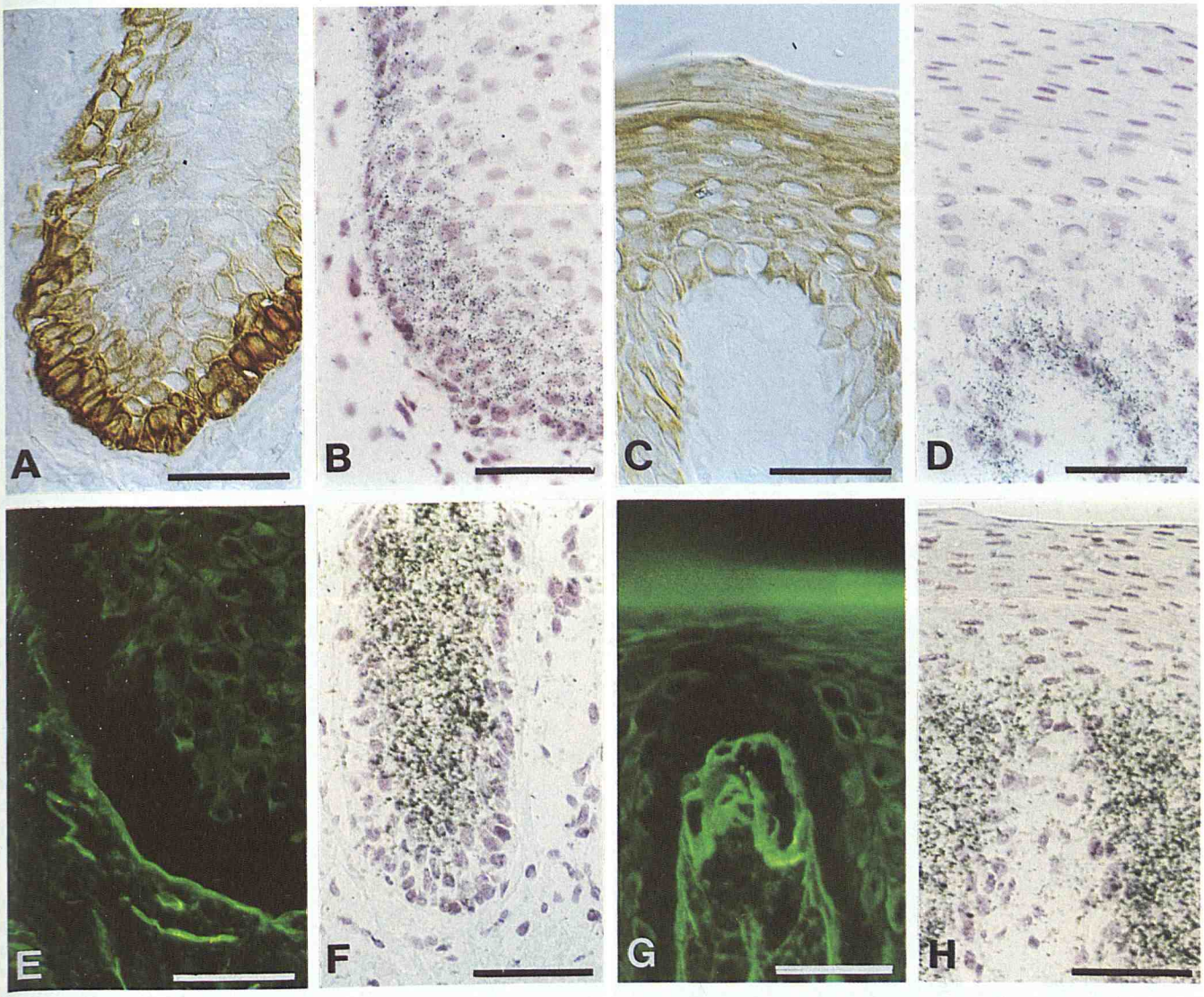

Figure 3. Different distribution in the center of psoriatic lesions of K5 and K10 mRNA, and K14 and K10 keratins in the rete ridges versus the juxtapapillary area. K14 keratin *(A)* and K5 mRNA *(B)* are detected in the rete ridges from the basal layer (strong staining) up to the second or third row of suprabasal cells (weaker staining). At the tip of the dermal papillas, K14 keratin is detectable in the totality of the epidermis although the staining is weak *(C)*. K5 mRNA *(D)* are localized mainly in the basal layer, but some labeling can be observed up to the third row of suprabasal cells. In the rete ridges, K10 keratin *(E)* appears only in the second or even the third suprabasal layer (note that the basement membrane is stained with anti-collagen-IV antibody). The pattern of distribution of K10 transcripts *(F)* is similar. At the tip of the dermal papillas, K10 mRNA *(H)* are weakly expressed in only few suprabasal cells whereas K10 keratin *(G)* is virtually absent. Note in the center of the figure the capillary loop, stained by the anti-collagen-IV antibody, which serves as a landmark of the dermal papilla. *Bar,* 50 μm.

proteins, contrary to what is seen in normal epidermis. In the center of the lesions, this delay was much more pronounced because K10 transcripts and K10 keratin appeared only several layers above the basal layer. The persistence of abundant K5 transcripts and K14 keratin throughout the epithelium also suggested that in these areas several layers of suprabasal keratinocytes remained undifferentiated.

We also found that the upper nucleated layers of psoriatic epidermis (parakeratotic stratum corneum) were devoid of K10 keratin messages and protein. Thus, this defect of expression in nucleated cells as well as the delayed onset of synthesis are probably both responsible for the previously reported reduction of suprabasal keratins in psoriasis [3–8].

As noted in the introduction, the presence in normal epidermis of basal keratinocytes already engaged in the differentiation process (transitional keratinocytes), although not yet fully acknowledged, is well documented [10–13]. Thus, we must admit that in normal epidermis, the stimulus triggering differentiation precedes the detachment of cells from the basement membrane. However, transitional keratinocytes are V-shaped and their staining with the basal-specific BP antibody is punctiform [12,13], suggesting that they are starting to detach from the basement membrane. The fact that the number of basal keratinocytes that are positive for K1/K10 messages is higher than the percentage positive for K1/K10 proteins [13] indicates that the commitment to differentiate precedes even more the detachment process. Transitional keratinocytes were also found to be actively proliferative [12]. This is not surprising, as proliferation only stops in suprabasal layers [23]. According to these results, neither detachment from the basement membrane nor proliferation arrest can be invoked as the stimulus triggering differen-

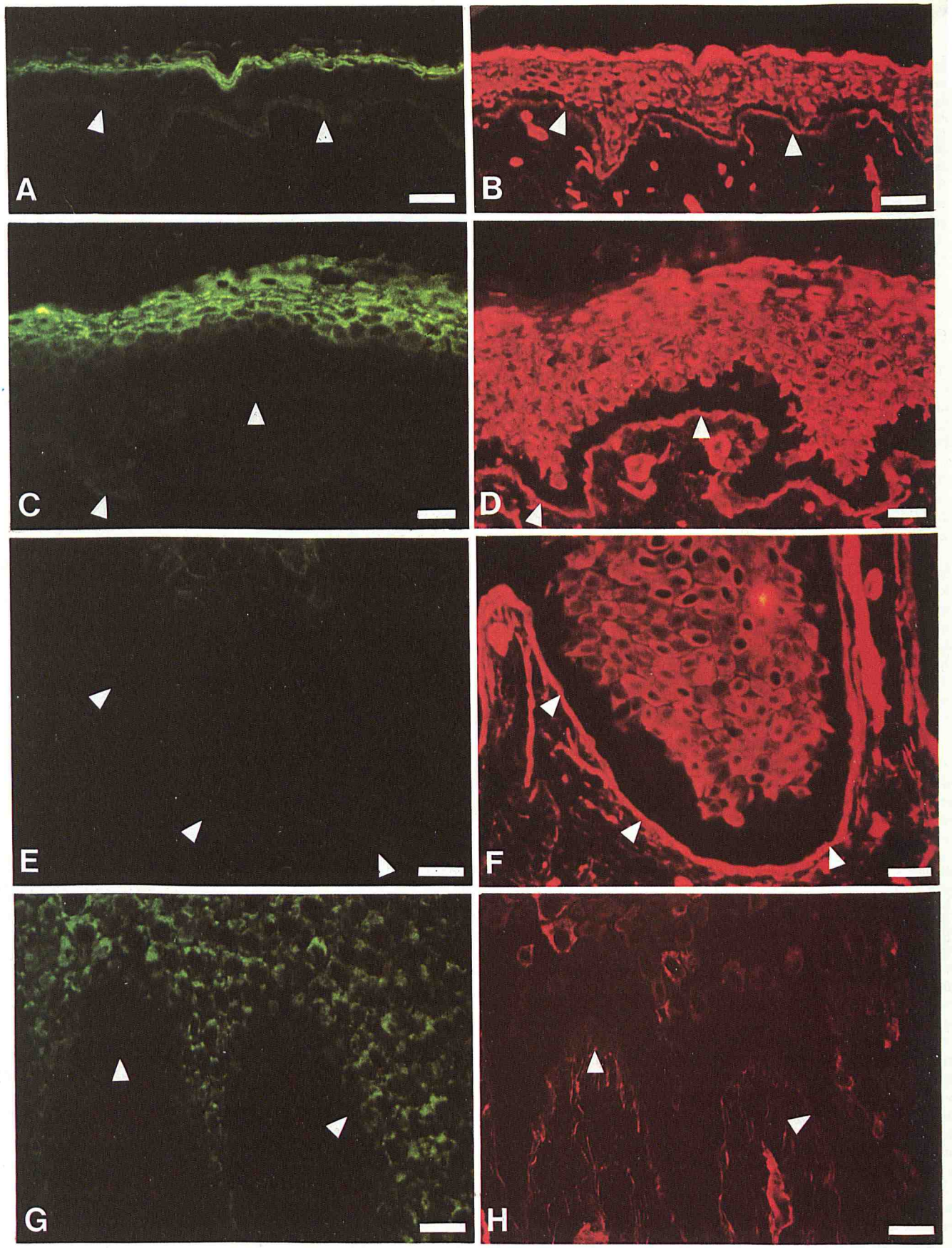

Figure 4. Compared distribution of involucrin and K10 keratin in normal and psoriatic epidermis. In normal human skin *(A,B)* involucrin *(A)* is expressed in granular layers, at the periphery of the cells. Immunolabeling with K10 keratin MoAb *(B)* shows mainly a suprabasal staining. *Bar,* 40 μm. At the periphery of psoriatic lesions *(C,D)*, involucrin *(C)* is detected in the spinous layers whereas K10 keratin is strictly suprabasal *(D)*. *Bar,* 40 μm. In the center of psoriatic lesions, in the rete ridge areas *(E,F)*, involucrin is distributed in the upper spinous layers, 5–6 rows above the basal layer, whereas K10 keratin is detected from 2–3 rows above the basal layer. In the juxtapapillar areas *(G,H)*, involucrin is detected from the first row of suprabasal cells up to the stratum corneum, whereas keratin K10 is barely detectable. The basement membrane zone *(triangles)* is labeled with collagen IV MoAb. *Bar,* 25 μm.

tiation of keratinocytes in normal epidermis. We cannot exclude that basal keratinocytes engage differentiation because of a stochastic process. If this were true, psoriatic keratinocytes should be considered as intrinsically abnormal, with a probability to engage in differentiation lower than that of normal keratinocytes. Alternatively, a specific stimulus such as the deprivation or the appearance of a hormone-like agent might trigger the differentiation process. In this context, the defect existing in psoriasis might be either an intrinsic property of the keratinocytes (their response being delayed) or a property of neighboring cells, not to say of cells of the endocrine or immune systems (the production of the agent being abnormal).

Studies by Weiss et al [8] and Stoler et al [4] support the idea that there is a mutual exclusiveness between expression of K1-K10 "keratinization-specific" keratins and K6-K16 "hyperproliferation-associated" keratins. Interestingly, because K6-K16 are also suprabasal [4], they appear as an alternative differentiation pathway [24]. Molecular studies at the level of keratin gene promoters should show whether transcription factors appearing during differentiation of keratinocytes are different in normal and hyperproliferative keratinocytes, and whether their specificities reflect the mutual exclusiveness seen at the mRNA and protein levels.

Although psoriasis is a highly pleiotropic condition, it is tempting to consider the biologic abnormalities, including those occurring in the last steps of differentiation, such as the decrease of filaggrin [2,3], and eventually the agranulosis and parakeratosis, as the consequences of one single early defect. From this point of view, it seems obvious that the investigation of the earliest defect that can be evidenced must be given priority. For the moment, the earliest sign of epidermal differentiation that has been detected is the appearance of "suprabasal keratins" [10-13,25-32], and we have shown that this appearance is delayed in psoriasis. But molecular markers other than K1 and K10 keratins also showing a heterogeneity in the basal layer remain to be found and studied in psoriasis, in particular markers that would discriminate between stem cells and transit amplifying cells [33].

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